REMARKS

The Examiner's comments in paragraphs 1-8 of the Office Action are acknowledged.

In response to the Examiner's comments in paragraphs 9-11 of the Office Action, claims 91 and 92 have been canceled without prejudice to the assertion of these claims in a duly filed divisional application.

In paragraph 12 of the Office Action, the Examiner suggested that the amendment which referenced the filing of a continuation application be deleted. This has been done in the present amendment.

The Examiner's courtesy in acknowledging the withdrawal of the objection of paragraphs 3-5 in the Office Action of February 12, 2003 and the rejections set forth in items 6a-c of the Office Action of February 12, 2003, in paragraphs 13 and 14 of the present Office Action is sincerely appreciated.

In paragraph 15 of the Office Action, claim 87 was rejected under 35 U.S.C.§112, second paragraph, for failing to particularly point out and distinctly claim the subject matter that the applicant regards as the invention.

Reconsideration is requested.

The provisions of 35 U.S.C.§112, second paragraph, provide that an applicant for a patent shall define his invention with reasonable precision in order to point out the invention. In the present case, the Examiner has questioned a time period of --48-200-- hours that appears in claim 87

because data has been presented that shows, under specific conditions, one experiment required a culture time of 96 hours to provide 95% of two chain uPA.

The applicant believes that a cultivation time of 48-200 hours particularly points out and distinctly claims the "subject matter which applicant regards as his invention" as required by Section 112, second paragraph. The single experiment reported in the Declaration was not intended and does not demonstrate that under all possible conditions and variables, it would always require 96 hours of culturing time to reach the claimed 95% level of uPA. For this reason, the Examiner's interpretation of that data in error. The purpose of the second paragraph of 35 U.S.C.§112 is to point out the invention. MPEP§2173.02 notes that in reviewing a claim for compliance with 35 U.S.C.§112, second paragraph, the Examiner must consider the claim as a whole to determine whether the claim apprises one of ordinary skill in the art of its scope and therefore serves its notice function. Since the claim before the Examiner clearly serves this function, it is requested that this ground of rejection be withdrawn.

In paragraph 16 of the Office Action, claims 81-90 were rejected under 35 U.S.C., first paragraph, because the specification while being enabling for a process as recited in claim 81 where the cell line is cultured for 96 hours, it does not enable a process as defined in claim 81 where the culturing time is less than 96 hours.

Reconsideration is requested.

The experiment reported in Fig. 2 of the Baici Declaration refers to the densitometric analysis of a single Western blot analysis of culture media collected at various times during a single production batch of human recombinant urokinase in the presence of butyrate. For this reason, the results are shown without their statistical significance.

The 95% value which is set forth in claim 81 represents an average value that has been measured to the precision that is permitted by the sensitivity of the analytical detection method used for its determination and is based on more than one experiment. Variations occur due to unavoidable experimental error and to the selection of biological systems as is well known to those who are skilled in the art.

The Applicant, who was well aware of this variability, disclosed a range of time in the specification during which on average will yield the indicated 95 % sc-uPA tc-uPA conversion in culture.

As far as values given in the Declaration are concerned, the Applicant based in his experience, believes that besides biological variability, the experiment in the Baici Declaration may have experimental errors due to pipetting errors, running the electrophoresis in a sodium dodecyl sulphate gel, electro-blotting to a membrane, staining with specific antibodies, and scanning the resulting Western blots by means of a densitometer. A major source of error in this type of experiment is the normal and inevitable broadening of the bands during electrophoresis and blotting. Considering additive variance, summation of the squared roots of the relative squared errors of each step yields the final error. Allowing for these expected sources of variance, anyone skilled in the art will agree that the assumption of a 5% variation in an experiment such as that shown in Fig. 2 of the Baici Declaration would conform to even the most rigorous statistical rules.

The Examiner's comments regarding the Baici Declaration where the 95 % conversion value is achieved in 96 hours did not acknowledge that 92% conversion was reached in 48 hours under the reported test conditions.

A 5% error applied to the 92% value as reported for 48 hours in the Baici Declaration would even place the 92% value within the 95% level of the claims.

The Applicant wishes to clarify that the experiment reported in Fig. 2 of the Baici Declaration is based on a densitometric analysis of a single Western blot analysis of culture media collected at various times during a single production batch of human recombinant urokinase in the presence of butyrate. Therefore results are shown without their statistical significance.

The 95% value, which characterizes the process of claim 81, represents an average value, which is precise as is permitted by the analytical detection method used for its determination.

The time range during which the specified average value of 95% of conversion is obtained, has been clearly indicated in the present specification and is set forth in days as being from 3 to 8 days (see p. 8, l. 23 to the end and page 9, l. 1-6) or in hours from 48 to 200, as follows:

> ``According to a preferred embodiment, the exhausted (i.e. the culture medium where cells have been grown) cell culture supernatant containing tc-uPA is recovered usually after 3-8 days in culture, usually at the fifth day when the balance between recombinant protein levels and cell viability (the latter kept preferably higher than 70%) is optimal. Alternatively the exhausted supernatant is recovered when sc-uPA is absent as

measured by SDS reducing PAGE, and maximally converted into tc-uPA, where for tc-uPA is intended a mixture of the HMW and LMW tc-uPA. Usually the optimal time for the recovery of mature tc-uPA is comprised between 48 and 200 hours, with a preferred time of 120 hours of culture in the presence of alkanoic acids or their derivatives or salts thereof and usually corresponds to a tc-uPA production level of about 4000 IU/ml. According to the described embodiments of the invention, conversion of the precursor forms (pre-prouPA, pro-uPA, scuPA) to the catalytically active tc-uPA is characterized by an efficiency higher than 95%, as determined by analytical reducing SDS-PAGE. Of the total tc-uPA produced, about 80% is in the HMW form and the remaining 20% is in the LMW form.''

For the sake of clarity, the time in hours was substituted for the definition in days. However the Applicant would like to emphasize that the differences in the value given in the Baici Declaration and the claimed 95% value is comprised within the range of 3% which is even lower than would be expected by the skilled person in experimental systems dealing in particular with biological applications.

MPEP§2164.08 points out that that the Examiner must consider the claim as a whole and not on the individual parts. Reasonable experimentation for the purpose of being able to practice an invention is allowed without causing a patent claim to be non-enabled.

MPEP§2164.08 also quotes from In re Goffe, 191 USPQ 429,431 (CCPA 1976):

"[T]o provide effective incentives, claims must adequately protect inventors. To

> demand that the first to disclose shall limit his claims to what he has found will work or to material which meet the guidelines for 'preferred materials' in a process such as the one herein involved would not serve the constitutional purpose of promoting progress in the useful arts."

It is well known that process conditions, materials and concentrations may be varied to influence the speed and yield of reactions. The disclosure of actual results at 96 hours does not *ipso facto* provide evidence that the process can not be accomplished in a greater or lesser period of time.

The Wands factors (In re Wands, 8 USPQ2d 731, 737 (Fed. Cir. 1988) have been discussed by the Examiner but the Examiner has not acknowledged that when the Wands decision is applied to the facts underlying the present rejection, a finding of proper enablement is required. The present case involves an operable experiment which is being used as the sole basis on which to argue that since the experiment required 96 hours, the application is not enabled for any other time period. In Wands, the applicant demonstrated that with regard to operability, 4 of 143 hybridomas were proved to fall within the claims and of those actually tested 4 of 10 proved to be operative. In the present case, the test that the Examiner is relying upon was within the claims and since this one test did not include all conditions and materials within the claims, there is no basis on which a skilled artisan would conclude that 96 hours is the minimum number of hours required to reach a yield of 95%. For this reason, the Examiner has not sustained his burden of showing that the disclosure is not

enabling for the claimed time range of 48-200 hours. For these reasons, it is requested that this ground of rejection should be withdrawn.

In paragraphs 17 and 18, the former rejections under 35 U.S.C.§102(b) and under 35 U.S.C.§103(a) were withdrawn in favor of the rejections of paragraphs 19 and 20.

In paragraph 19 of the Office Action, claims 81-85 and 87-89 were rejected under 35 U.S.C.§103(a) as being unpatentable over Okabayashi et al. (Okabayashi) in view of the state of the art as represented by Zang et al.(Zang).In paragraph 20 of the Office Action claims 86 and 90 were rejected over the Okabayashi and Zang references further in view of Anderson et al. (Anderson).

Reconsideration is requested.

The present invention provides a process not for the purpose of increasing the total amount of urokinase produced by genetically engineered cell, but a process leading to the production of two chain urokinase in an almost purified form, i.e. of an industrially applicable process leading to the production of recombinant urokinase in its activated form (tc-uPA).

This achievement, as will be demonstrated herein, has never been disclosed prior to the present invention (see present patent specification , page 2, lines 5-22):

> ``Various attempts in the production of tc-uPA by the recombinant DNA technologies confirm on one side the relevance of such a molecule in the clinic and on the other side the need of such an approach required mainly for safety and purity reasons. EP 154272 describes

the production of recombinant glycosylated scuPA obtained by insertion of the cDNA sequence in animal cells.EP 303028 describes the production of recombinant glycosylated scuPA obtained by insertion of the genomic sequence into mammalian cells. The production described in these patents refers to recombinant Urokinase (sc-uPA) in the enzymatically inactive form.

Production of the active enzyme (tc-uPA) by recombinant DNA is still an open question, mainly because of its complex extracellular processing. As a matter of fact, low amounts of recombinant active tc-uPA is obtained in some of the recombinant eukaryotic systems described so far. In these eukaryotic systems the resulting product is a mixture of sc-uPA and tc-uPA (Cheng D et al., Chinese Journal of Biotechnology, 1994, 9: 151-159). This is mainly due to the inefficiency of the processing steps involved in sc-uPA activation This fact raises some problems for the purification of the two individual forms.''

It is known in the prior art that, sc-uPA and tc-uPA are different molecules even though the latter is derived by proteolytic processing from the former. The former is a unique polypeptide appearing as a single band, running at about 55kD on a reducing SDS-PAGE, the latter is made up of two chains, respectively running at about 19kD and 34kD. This is clearly indicated in the specification, Background Art discussion (see page.1, lines. 19-32

''Within the blood plasmin performs a proteolytic cleavage which converts the proenzyme sc-uPA into a two-chain polypeptide, named tc-uPA, which is catalytically and physiologically active. tc-uPA is composed of an A- and a B-chain, linked to each other by a disulfide bond''.

The disappearance of the single higher MW band and the appearance of the two lower MW bands after butyrate treatment has been also demonstrated by Western-blot on fig. 1 of the specification.

Therefore, sc-uPA and tc-uPA are not just two ways of defining the same molecule. This is clearly confirmed by differences in their physiological role, different substrate affinities and, of course, pharmacological properties etc.

In view of this clarification, aimed at further stressing the difference between active and inactive uPA, or between tc-uPA and sc-uPA, already pointed out in the Application, and in view of the various attempts of recombinant urokinase production, the Applicant respectfully asks that production of active urokinase, i.e. of tc-uPA as presently claimed in claim 81, would not be considered inherent to the process for the production of sc-uPA, even though in the prior art, the two molecules have been confused and comprised under the generic term of urokinase.

On the contrary, the present invention, which allows the recovery of an almost pure tc-uPA (i.e up to a level of at least 95%), represents an important achievement for the therapeutic use of said enzyme.

In fact, purification of a single molecular species, i.e. tc-uPA, in prior art processes where the conversion, if any, of the inactive to the active form is not so efficient, becomes extremely laborious and economically not advantageous.

The rejection for obviousness that has been raised by the Examiner over Okabayashi in view of Zang is in error for the following reasons:

- Okabayashi describes a process for increasing the overall production of recombinant urokinase in CHO cells by the addition of butyrate for a time not longer than 24 hours.

Conclusions drawn by Author in the final remarks are that: ``Butyrate treatment increased not only the extracellular level but also the intracellular urokinase.'' (see abstract, last sentence).

Nothing else can be derived from the disclosure of Okabayashi because the assay therein described does not discriminate between the production of catalytically active or catalytically inactive form and is therefore unable to suggest the invention as disclosed in the present Application.

The assay used in Okabayashi does not allow a skilled worker in the art to retrieve any further information because the urokinase is detected in the presence of an activator of uPA, as noted at page 582, 1st line:

> ``...This assay involves the use of an agar plate containing plasminogen and fibrin; when a sample containing a plasminogen activator is added to a well in the agar, the plasminogen is converted to the active enzyme plasmin.''

It is requested that the Examiner is note that since the assay mixture contains plasminogen, which is the precursor of an activator of urokinase, the plasminogen may be converted to plasmin by even small amounts of active urokinase, and:

1) there is no possibility of deriving any information about the amount of catalytically active uPA originally present in the cell culture, as the total amount of sc-uPA is, in any case, converted to tc-uPA during the assay; moreover,
2) any distinction between tc-uPA and sc-uPA is not found within the disclosure of the Okabayashi paper which is rather focused to a method to increase the overall production of uPA, as confirmed by the fact that this increase is measured also at the intracellular level upon an unprocessed protein.

Therefore the disclosure of Okabayashi does not make obvious the claimed process because it does not disclose a method which meets all the features defined in claim 81 (addition of butyrate or analogues for a time comprised form 48 to 200 hours) but most importantly, fails completely to show or to suggest results achieved only in the present invention (obtaining a yield of 95 % tc-uPA).

Zang only discloses CHO culture conditions which allow for the production of two recombinant proteins, urokinase and a humanized immunoglobulin in a protein-free medium for different incubation times.

The production of urokinase is evaluated during a period of 14 weeks (see figure 2 and page 390, 2nd col. half page).

Conclusions drawn by the Zang are that (see page 391, 1st col. last and penultimate sentence):

``The CHO SSF3 cells are well suited to scale up and can be adapted to good growth and productivity under protein free conditions. ...the successful application of these cells for the secretion of recombinant uPA and IgG LC at a substantial level using protein-free media should extend their utility for other research applications and production purposes.''

Again, as it is derived from the molecular weight shown in Fig. 4, the urokinase produced at the end of this process, is the single chain and inactive form of urokinase (sc-uPA) (see fig. 4, p. 391).

Therefore, the combination of Okabayashi with Zang can only suggest a process which comprises the addition of butyrate and the incubation of the culture for a time comprised from 5 days to 14 weeks. A combination possible, however, merely to achieve a stable or an increased production of (mainly inactive) urokinase, as stated also by the Examiner

on page 11 of the Office Action and not to achieve the result of the invention.

The combination of references fails to suggest all the features of claim 81, in particular the incubation time comprised from 48 to 200 hours.

In addition, contrary to the Examiner's allegation, the Applicant respectfully submits that the motivation to combine the Okabayashi and the Zang documents is untenable for the following reason:

- on page 585, 4th line to the end, Okabayashi notes that by adding butyrate for recombinant protein production, contradictory results have been obtained and provides a number of references. He concludes as follows:

  ``We have shown that butyrate activates the expression of foreign genes directed by the SV40 early region promoter in recombinant CHO-K1 cells. Since CHO-K1 cells have been widely used as a recipient for DNA mediated gene transfer, this treatment displays a strong potential for widespread application. Its applicability, however, should be carefully evaluated since butyrate inhibits cell growth.''.

As confirmed by Okabayashi, inhibition of cell growth by butyrate was something already known by the skilled in the art at the time of filing of the present Application. It was known in fact that the inhibitory effect of butyrate was due by an arrest of the cell cycle in G1

The skilled artisan, fully aware of the possible detrimental effect of butyrate on cell growth, would have not chosen to incubate this potentially toxic substance (butyrate) on the cells for a time longer than necessary i.e. for a time longer that disclosed in Okabayashi, to achieve an increase in urokinase production.

As a matter of fact, Okabayashi describes the effect on urokinase production as ``rapid'' (see page 583, last sentence): (``The response was rapid: an effect on urokinase production was observed as early as 6 hours after exposure to butyrate''). Even though in Okabayashi butyrate was added for a time as long as 24 hours to the culture medium, the overall teaching drives away from trying an incubation time longer than 24 hours.

In other words the skilled artisan would have been directed away from combining the teaching of Okabayashi and the teaching of Zang with the purpose of increasing the production of recombinant single chain urokinase, let alone to achieve the production of active two chain urokinase.

Okabayashi carried out their cultivation experiments in the presence of butyrate exclusively to achieve an increased productivity of Urokinase (no discrimination between sc- and tc-uPA, as already demonstrated), whereas Zang perform the same experiment with a different medium (serum-free, 5 days to 14 weeks cultivation time) in the absence of butyrate. Because of the above mentioned and known toxic effects of butyrate on mammalian cells, it is not obvious to directly combine the cited references with the purpose of achieving activation of u-PA because there is no teaching to carry out such a process. The aims of the two different references are exclusively focused on increasing the recombinant urokinase productivity.

For the sake of completeness, we recall that also Zang et al. describe the production of a ``single chain form'' of urokinase (see page 390, rows 49-50, and fig 4), with absolute no insight on the possibility to achieve activated urokinase.

In view of the above the Applicant, further remarks that no motivation could bring the skilled artisan to combine the cited documents. On the contrary a strong prejudice against

their combination is found in the common general knowledge, as confirmed by Okabayashi in the sentence reported above.

Therefore the Applicant respectfully submits that the skilled artisan would not *a priori* expect the result obtained in the present Application, by a process derived from the untenable combination of the Okabayashi and the Zang documents; let alone the skilled man would have obtained the production of urokinase in its active form up to at least 95%, by combining disclosures aimed at increasing only an overall production of this recombinant enzyme, completely silent about its degree of activation.

In conclusion, the Applicant believes that the obviousness objections have based on hindsight and are not tenable.

The same applies on the Examiner's comment on the inherence of the production of active tc-uPA in Okabayashi: this feature is not inherent to the disclosure of Okabayashi, but has been derived by the Examiner from the Baici Declaration, in particular on the basis of the comparative data for the level of tc-uPA production after 24 hours of butyrate incubation.

The Examiner further alleges that Anderson teach a process for the cultivation of CHO cells for the recombinant production of urokinase. The Applicant respectfully disagrees with the Examiner because Anderson discloses a process for the production of recombinant tissue type plasminogen activator (t-PA).

Although t-PA and urokinase (u-PA) share some physiological functions, nevertheless they are two completely distinct proteins with their peculiar chemical composition that distinguishes two different chemical entities.

Nothing regarding the possible activation of the t-PA molecule is reported, and, of course, uPA is not even mentioned.

Again, even for someone skilled in the art it would be difficult, if not misleading to apply the information disclosed in Anderson to a process focused to the production of active u-PA. For these reasons, it is requested that the grounds for rejection be withdrawn.

An early and favorable action is earnestly solicited.

Respectfully submitted,

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